

Marked Up Version of Sequence Listing After Amendment of September 5, 2002

SEQUENCE LISTING

<110> Eck, Jorgen
Schmidt, Arno
Zinke, Holger

<120> Recombinant Fusion Proteins Based on
Ribosome-Inactivating Proteins of the mistletoe Viscum
album

<130> 09282-5

<140> 09/347,064

<141> 1999-07-02

<150> PCT/EP98/00009

<151> 1998-01-02

<150> EP 97 10 0012.0

<151> 1997-01-02

<160> 38

<170> PatentIn Ver. 2.1

<210> 1

<211> 762

<212> DNA

<213> Viscum album

<400> 1

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ctcttgctgc agtctacgat ccccgctctcc gatgcgcaaa gatttgtctt ggtggagctc 180
accaaccagg ggggagactc gatcacggcc gccatcgacg ttaccaatct gtacgtcgtg 240
gcttaccagg caggcgacca atcctacttt ttgcgcgacg caccacgcgg cgcggaacg 300
catctcttca ccggcaccac ccgatcctct ctccattca acggaagcta ccctgatctg 360
gagcgatacg ccggacatag ggaccagatc cctctcggta tagaccaact cattcaatcc 420
gtcacggcgc ttcggtttcc gggcggcagc acgcgtaccc aagctcgttc gattttaatc 480
ctcattcaga tgatctccga ggccgccaga ttcaatccca tcttatggag ggctcgccaa 540
tacattaaca gtggggcgtc atttctgcca gacgtgtaca tgctggagct ggagacgagt 600
tgggggccaac aatccacgca agtccagcat tcaaccgatg gcgtttttta taaccaatt 660
cggttggcta tcccccccg taacttcgtg acgttgacca atgttcgcga cgtgatcgcc 720
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<210> 2

<211> 252

<212> PRT

<213> Viscum album

<400> 2

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Met Tyr Glu Arg Ile Arg Leu Arg Val Thr His Gln Thr Thr Gly Glu
  1           5           10          15
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Glu Tyr Phe Arg Phe Ile Thr Leu Leu Arg Asp Tyr Val Ser Ser Gly
          20          25          30
```

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Ser Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val
      35              40              45

Ser Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly
      50              55              60

Asp Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala
      65              70              75              80

Tyr Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly
      85              90              95

Ala Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe
      100              105              110

Asn Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln
      115              120              125

Ile Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg
      130              135              140

Phe Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu
      145              150              155              160

Ile Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg
      165              170              175

Ala Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr
      180              185              190

Met Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln
      195              200              205

His Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro
      210              215              220

Pro Gly Asn Phe Val Thr Leu Thr Asn Val Arg Asp Val Ile Ala Ser
      225              230              235              240

Leu Ala Ile Met Leu Phe Val Cys Gly Glu Arg Pro
      245              250

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<210> 3

<211> 828

<212> DNA

<213> Viscum album

<400> 3

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aggcctgtga tagccgatga tggtacatgt agtgcttcgg aacctacggt gcggattgtg 60
ggtcgaaatg gcatgtgcgt ggacgtccga gatgacgatt tccgcatgg aaatcagata 120
cagttgtggc cctccaagtc caacaatgat ccgaatcagt tgtggacgat caaaagggat 180
ggaaccattc gatccaatgg cagctgcttg accacgtatg gctatactgc tggcgtctat 240
gtgatgatct tcgactgtaa tactgctgtg cgggaggcca ctctttggca gatatggggc 300
aatgggacca tcatcaatcc aagatccaat ctggttttgg cagcatcatc tggaatcaaa 360
ggcactacgc ttacggtgca aacactggat tacacgttgg gacagggctg gcttgccggt 420

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aatgataccg cccacgcga ggtgaccata tatgggttca gggacctttg catggaatca 480
aatggaggga gtgtgtgggt ggagacgtgc gtgagtagcc aaaagaacca aagatgggct 540
ttgtacgggg atggttctat acgccccaaa caaaaccaag accaatgcct cacctgtggg 600
agagactccg tttcaacagt aatcaatata gttagctgca gcgctggatc gtctgggcag 660
cgatgggtgt ttaccaatga aggggccatt ttgaatttaa agaatgggtt ggccatggat 720
gtggcgcaag caaatccaaa gctccgccga ataatcatct atcctgccac aggaaaacca 780
aatcaaatgt ggcttcccg ggcaggtgga taccactagt aaggatcc 828

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<210> 4
 <211> 267
 <212> PRT
 <213> Viscum album

<400> 4
 Asp Asp Val Thr Cys Ser Ala Ser Glu Pro Thr Val Arg Ile Val Gly
 1 5 10 15
 Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
 20 25 30
 Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
 35 40 45
 Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
 50 55 60
 Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
 65 70 75 80
 Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
 85 90 95
 Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
 100 105 110
 Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
 115 120 125
 Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
 130 135 140
 Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
 145 150 155 160
 Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
 165 170 175
 Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
 180 185 190
 Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys
 195 200 205
 Ser Ala Gly Ser Ser Gly Gln Arg Trp Val Phe Thr Asn Glu Gly Ala
 210 215 220

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Ile Leu Asn Leu Lys Asn Gly Leu Ala Met Asp Val Ala Gln Ala Asn
225 230 235 240

Pro Lys Leu Arg Arg Ile Ile Ile Tyr Pro Ala Thr Gly Lys Pro Asn
245 250 255

Gln Met Trp Leu Pro Val Pro Gly Gly Tyr His
260 265

<210> 5
<211> ~~48~~ 72
<212> DNA
<213> Viscum album

<400> 5
cgcccgagtt cctctgaggt gcgctattgg ccgctggcca taaggcctgt gatagccgat 60
gatgttacat gt 72

<210> 6
<211> ~~16~~ 17
<212> PRT
<213> Viscum album

<400> 6
Ser Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile
1 5 10 15
Ala

<210> 7
<211> 756
<212> DNA
<213> Viscum album

<400> 7
tacgaacgta tccgtctgcg tgttaccac cagaccaccg gtgaagaata tttccgggttc 60
atcacgcttc tccgagatta tgtctcaagc ggaagctttt ccaatgagat accactcttg 120
cgtcagtcta cgatccccgt ctccgatgcg caaagatttg tcttggtgga gtcaccaaac 180
caggggggag actcgatcac ggccgccatc gacgttacca atctgtacgt cgtgggttac 240
caagcaggcg accaatccta ctttttgcg gacgcaccac gcggcgcgga aacgcatctc 300
ttcaccggca ccaccgatc ctctctccca ttcaacggaa gctaccctga tctggagcga 360
tacgccggac atagggacca gatccctctc ggtatagacc aactcattca atccgtcacg 420
gcgcttcggt ttcggggcgg cagcacgcgt acccaagctc gttcgatttt aatcctcatt 480
cagatgatct ccgaggccgc cagattcaat cccatcttat ggagggtctg ccaatacatt 540
aacagtgggg cgtcatttct gccagacgtg tacatgctgg agctggagac gagttggggc 600
caacaatcca cgcaagtcca gcattcaacc gatggcggtt ttaataaacc aattcggttg 660
gctatacccc ccggttaact cgtgacgttg accaatgttc gcgacgtgat cgccagcttg 720
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<210> 8
<211> 252
<212> PRT
<213> Viscum album

<400> 8

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Tyr Glu Arg Ile Arg Leu Arg Val Thr His Gln Thr Thr Gly Glu Glu
 1           5           10           15

Tyr Phe Arg Phe Ile Thr Leu Leu Arg Asp Tyr Val Ser Ser Gly Ser
          20           25           30

Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val Ser
          35           40           45

Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly Asp
          50           55           60

Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala Tyr
          65           70           75           80

Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly Ala
          85           90           95

Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe Asn
          100          105          110

Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln Ile
          115          120          125

Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg Phe
          130          135          140

Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu Ile
          145          150          155          160

Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg Ala
          165          170          175

Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr Met
          180          185          190

Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln His
          195          200          205

Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro Pro
          210          215          220

Gly Asn Phe Val Thr Leu Thr Asn Val Arg Asp Val Ile Ala Ser Leu
          225          230          235          240

Ala Ile Met Leu Phe Val Cys Gly Glu Arg Pro Ser
          245          250

```

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<210> 9
<211> 789
<212> DNA
<213> Viscum album

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<400> 9
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tgcgtggacg tccgagatga cgatttccgc gatggaaatc agatacagtt gtggccctcc 120

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aagtcacaaca atgatccgaa tcagttgtgg acgatcaaaa gggatggaac cattcgatcc 180
aatggcagct gcttgaccac gtatggctat actgctggcg tctatgtgat gatcttcgac 240
tgtaatactg ctgtgcggga ggccactctt tggcagatat ggggcaatgg gaccatcatc 300
aatccaagat ccaatctggt tttggcagca tcactctggaa tcaaaggcac tacgcttacg 360
gtgcaaacac tggattacac gttgggacag ggctggcttg ccggtaatga taccgccccca 420
cgcgaggtga ccatatatgg gttcagggac ctttgcattg aatcaaatgg agggagtgtg 480
tggttgagaga cgtgcgtgag tagccaaaag aaccaaagat gggctttgta cggggatggt 540
tctatacgcc ccaaacaaaa ccaagaccaa tgcctcacct gtgggagaga ctccgtttca 600
acagtaatca atatagttag ctgcagcgct ggatcgtctg ggcagcgatg ggtgtttacc 660
aatgaagggg ccattttgaa tttaaagaat gggttggcca tggatgtggc gcaagcaaat 720
ccaaagctcc gccgaataat catctatcct gccacaggaa aaccaaataa aatgtggctt 780
cccgtgccca                                     789

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<210> 10

<211> 263

<212> PRT

<213> Viscum album

<400> 10

```

Asp Asp Val Thr Cys Ser Ala Ser Glu Pro Thr Val Arg Ile Val Gly
  1                      5                      10                      15

```

```

Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
      20                      25                      30

```

```

Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
      35                      40                      45

```

```

Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
      50                      55                      60

```

```

Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
      65                      70                      75                      80

```

```

Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
      85                      90                      95

```

```

Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
      100                      105                      110

```

```

Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
      115                      120                      125

```

```

Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
      130                      135                      140

```

```

Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
      145                      150                      155                      160

```

```

Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
      165                      170                      175

```

```

Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
      180                      185                      190

```

```

Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys

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195 200 205
 Ser Ala Gly Ser Ser Gly Gln Arg Trp Val Phe Thr Asn Glu Gly Ala
 210 215 220
 Ile Leu Asn Leu Lys Asn Gly Leu Ala Met Asp Val Ala Gln Ala Asn
 225 230 235 240
 Pro Lys Leu Arg Arg Ile Ile Ile Tyr Pro Ala Thr Gly Lys Pro Asn
 245 250 255
 Gln Met Trp Leu Pro Val Pro
 260

 <210> 11
 <211> 48
 <212> DNA
 <213> Viscum album

 <400> 11
 tcctctgagg tgcgctattg gccgctggtc atacgaccgc tgatagcc 48

 <210> 12
 <211> 16
 <212> PRT
 <213> Viscum album

 <400> 12
 Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile Ala
 1 5 10 15

 <210> 13
 <211> 94
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:Synthetic gene
 encoding amino acids 53-78 of human P2 protein

 <400> 13
 gtaccgggtg gcggtcgtag cgaatccacc ttcaaaaaca ccgaaatctc cttcaaactg 60
 ggtcaggaat tcgaagaaac caccgctgac aact 94

 <210> 14
 <211> 26
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:Amino acids
 53-78 of human P2 protein

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<400> 14
Arg Thr Glu Ser Thr Phe Lys Asn Thr Glu Ile Ser Phe Lys Leu Gly
1 5 10 15

Gln Glu Phe Glu Glu Thr Thr Ala Asp Asn
20 25

<210> 15
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 20:
Synthetic linker cassette for providing modularity
at the 3' end of rMLB delta 1alpha 1beta

<400> 15
cacgggtaaa ccgaaccaga tgtggctgcc ggtaccgtag taacgctcct cgtcgaccta 60
gtaaggatcc ctgga 75

<210> 16
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 20: amino
acid sequence encoded by portion of SEQ ID NO: 15

<400> 16
Thr Gly Lys Pro Asn Gln Met Trp Leu Pro Val Pro
1 5 10

<210> 17
<211> 82
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 21:
Synthetic linker cassette for providing modularity
at the 3'end of rMLB Delta 1alpha 1beta 2gamma
with affinity module ("His-Tag").

<400> 17
ccggtaaacc gaaccagatg tggctgccgg taccgggtgg tggatatcat caccaccatc 60
accactagta actcctcgga tc 82

<210> 18
<211> 21
<212> PRT
<213> Artificial Sequence

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<220>
<223> Description of Artificial Sequence:Amino acid
sequence encoded by a portion of SEQ ID NO: 17

<400> 18
Gly Lys Pro Asn Gln Met Trp Leu Pro Val Pro Gly Gly Gly Tyr His
1 5 10 15
His His His His His
20

<210> 19
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Codon exchange
rMLB D23A

<400> 19
catgtgcgtg gccgtccgag atgacg 26

<210> 20
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1alpha2
(W38A). -

<400> 20
cagatacagt tggcgccctc caagtcc 27

<210> 21
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1beta (Y68S,
Y70S, Y75S, F79S). -

<400> 21
gctgcttgac cacgtctggc tctactgctg gcgtctctgt gatgatctcc gactgtaata 60
c 61

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<210> 22
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1beta1
(D235A). -

<400> 22
gggttgcca tggctgtggc gcaagc

26

<210> 23
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 2gamma2
(Y249A). -

<400> 23
cgaataatca tcgctcctgc cacagg

26

<210> 24
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - pT7 EcoRV to
SspI. -

<400> 24
cttccttttt caatattatt gaagcattta tcagg

35

<210> 25
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - pT7 SspI to
EcoRV. -

<400> 25

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• ccttcctttttt cgatatcatt gaagcattta tcagg

35

<210> 26
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - pT7 Delta NdeI to
StuI. -

<400> 26
ctttaagaag gagatataca ggcctacgag aggctaagac

40

<210> 27
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLB silent NheI. -

<400> 27
gttacctgca gtgctagcga acctacgggtg cgg

33

<210> 28
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLA Delta AgeI. -

<400> 28
cccaccagac caccggcgaa gaatatttcc gg

32

<210> 29
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes.

<400> 29

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ggttgatgc ggagagcgtc cctcgagctc tgagggtcgc

40

<210> 30
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLB Delta EcoNI to
AgeI. -

<400> 30
ccgaataatc atcgctccgg ccaccggtaa accaaatcaa atg

43

<210> 31
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Flanking region
of the ProML gene cassette in expression vector
pT7ProML

<400> 31
tacatatgta c

11

<210> 32
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Flanking region
of the ProML gene cassette in expression vector
pT7ProML

<400> 32
ccatgataag gatcctctag

20

<210> 33
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Flanking region
of the IML gene cassette in expression vector
PIML-02-P

<400> 33

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'cāggcctac

9

<210> 34
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Flanking region
of the IML gene cassette in expression vector
PIML-02-P

<400> 34
cactagtaac tcctcggatc ctctagagtc gacc

34

<210> 35
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Modulator
module peptide

<400> 35
Lys Asp Glu Leu
1

<210> 36
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Modulator
module peptide

<400> 36
His Asp Glu Leu
1

<210> 37
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Portion of the
ML propeptide

<400> 37
Ser Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile
1 5 10 15

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Ala

<210> 38

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: A degradation
product of myelin basic protein.

<400> 38

Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro
1 5 10